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Report of the Shipboard Verification tests of the ballast water management system of Coldharbour Marine Ltd.

SB-1401-Coldharbour

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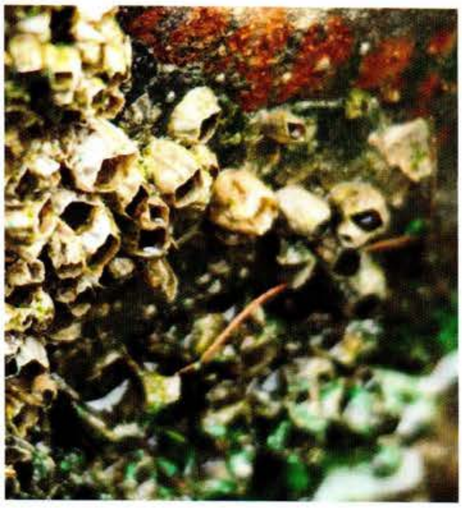
Water and sediment of ships should be free of invasive organisms



"There is no wisdom without ballast"

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TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

On behalf of Coldharbour Marine Ltd (further referred as Coldharbour), a total of six test runs were performed on board the VLCC *Alfa Glory* in the period March until October 2014. The purpose of those tests was to verify the performance during ship-board tests of their ballast water treatment system (BWTS), in accordance with the IMO Ballast Water Management Convention (Anon., 2004) and the Guideline G8 (Anon., 2008).

Tests were generally conducted in accordance with the Test protocol as previously established by MEA-nl and Coldharbour and verified by Lloyd's Register on behalf of the Maritime & Coastguard Agency of the United Kingdom.

Six test runs were performed. Three test runs were successfully completed and proved to be in compliance with Regulation D-2. Two test runs could not be completed in accordance with the requirements due to ship's operations. One test run was invalid due to technical problems of the vessel.

Table 1: Dates and location of intake and discharge

Testrun	Intake	Location	Discharge
SI	29.03.14	Singapore harbour	na
SII	01.06.14	Singapore anchorge	11.06.14
SIII	18.06.14	SW Chinese Sea	na
SIV	25.08.14	SW of Sri Lanka	03.09.14
SV	12.10.14	Gulf of Oman	18.10.14
SVI	12.10.14	Gulf of Oman	18.10.14
na = not applicable			

In conclusion, the tests showed that the BWTS of Coldharbour is capable of operating successfully during shipboard tests on a VLCC, in accordance with the requirements of the IMO Ballast Water Management Convention (Anon., 2004).

Table 2: Summary of relevant results

	Intake Treated t0			Discharge Treated tX		
	Average	Min	Max	Average	Min	Max
plankton > 50 µm [counts/m³]	11,519.4	8,325.0	13,483.3	2.7	0.0	6.7
phytoplankton 2 - 50 µm [counts/ml]	622	121	1,581	< 10	< 10	< 10
microzooplankton 10-50 µm [counts/ml]	0.74	0.60	0.87	0.27	0.04	0.43
total bacteria [counts/ml]	1.3E+06	7.2E+05	1.6E+06	6.0E+05	4.5E+05	7.0E+05
E. coli [cfu/ml]	< 1	< 1	< 1	< 1	< 1	< 1
Enterococci [cfu/100 ml]	< 1	< 1	< 1	< 1	< 1	< 1
V. cholerae	< 1	< 1	< 1	< 1	< 1	< 1

Summary table covering all biological results required for shipboard verification tests in Guideline G8 (Anon. 2008; Annex 4, Part 2, Section 2.1).

2. INTRODUCTION

Commissioned by Coldharbour Marine Ltd. (Further Coldharbour), MEA-nl BV (further MEA-nl) performed ship-board tests on their ballast water treatment system (BWTS).

Between March 28th and October 20th, 2014 a total of six independent test runs were conducted on board VLCC *Alfa Glory* (Panama flag, built 1997, IMO number 9108154, a deadweight of 309,636 mt and a ballast capacity of 101,642.4 m^3). Purpose of the tests was to assess the performance of the BWTS according to the IMO Ballast Water Management Convention (Anon., 2004) (further the Convention). This report describes more specific the tests according to the criteria of Annex 4 to the Guideline G8 (Anon., 2008), section 2.2, Ship-board tests.

To ensure proper control of the processes, MEA-nl has an ISO-9001:2008 certified management system (**Annex I**). The tests were conducted in accordance with the mutual agreed test protocol (Quality Plan) (MEA-nl, 2014). During the test runs some deviations of the test protocol were necessary. Where applicable, this was discussed amongst MEA-nl, Coldharbour and for approval with Lloyd's Register. Details of deviations and evaluation of the possible influence on the test results will be discussed at the relevant test run in **Chapter 3**.

Tests were witnessed by Lloyds' Register as the recognized organisation (RO), acting on behalf of the UK Maritime & Coastguard Agency (MCA), being the national administration for Type Approval.

A description of the system, including its relevant particulars, is given in the test protocol. For a general understanding, it is good to know that the system treats water whilst in the particular ballast tank (in-tank treatment), where the majority of systems treats water at intake and/or discharge (in-line treatment).

The time span of the tests is in accordance with the duration requirements as described in Guideline G8 of IMO (paragraph 2.2.2.7 of the Annex; Anon., 2008).

Water quality of the challenge water at intake for all six test runs can be described as tropical-oceanic. Despite the intake location being in the harbour of Singapore, intake conditions for test runs SI and SII were best described as oceanic. The intake location for test runs SV and SVI was in the Gulf of Oman, which is located at the northern border of the tropical region. As can be concluded from the data, water conditions were best described as tropical (**Chapter 4**).

In order to assess the system's performance rather than external factors, the impact of the ship's operation on the system have to be taken into consideration. This became especially apparent during test run SIV, where the TSS load in the tank for control water was significantly different from the tank for treated water at intake.

This report only documents the biological performance of the Coldharbour BWT system and not the technical performance, nor settings of the BWT system, other than where it affected the analytical procedures performed by MEA-nl. The detailed report of the technical performance of the system during ship-board tests is documented in a separate report by Coldharbour.

3. GENERAL INFORMATION ON EACH TEST RUN

Test runs were numbered starting with an "S" and in roman numerals from I to VI. Replicate samples were numbered 1 to 3. Ballast-tanks of the ship are numbered 1 to 5 portside (PS) and starboard (SB), but not all were used for testing. Both ballast tanks number 4 PS and SB were equipped with the treatment system and used as treated tanks for all six test runs. Ballast tanks number 2 PS and SB were used as control tanks for all tests. Volume of ballast tanks number 4 was 9,200 m^3 each. Volume of ballast tanks number 2 was 9,500 m^3 each.

Two of the six test runs performed, number SI and SIII, were not complete test runs, but were cancelled after intake. Test run SIV was invalid due to a technical failure of the ship. Ship's generators could not provide power to BWT installation. Test runs SII, SV and SVI were technically completed successfully. With the omission of test runs SIII and SIV, because of factors not related to the BWMS, the remaining three test runs (SII, SV & SVI) comply with the requirement of three consecutive, successful test runs (paragraph 2.2.2.8 of the Annex to G8; Anon., 2008).

Test runs SII, SIII, SV and SVI, were witnessed by surveyors from Lloyds' Register. Test runs SII and SIII by Mr. Theo from the Malaysian office. The discharge of test runs SV and SVI was witnessed by Mr. Klimenko from the Bahrain office. Both surveyors reported their findings in separate reports, not available to MEA-nl.

3.1 Test run SI

This first intake was done in Singapore harbour, during cargo discharge operations. The vessel discharged its cargo directly to the production line of the receiving oil-refinery, resulting in a slow offload rate. In turn, this caused the ballast water uptake to be spread over three days (March 29th – 31st). Total ballast pumping time was 59 hours, with several breaks in between. Samples were taken on all three days in order to representatively cover the intake of challenge water over the whole period.

As mentioned above, this intake was not followed by a treatment and subsequent discharge. This was due to the vessel's operation. Vessel was ordered to proceed to off port limits and wait for orders. Crew was reduced to skeleton crew only and all supernumeraries were disembarked.

The biological data collected on intake formed the basis for two deviations from the initial test protocol, implemented after consultation and approval with the RO.

Firstly the extension of the size class of 10 – 50 micron (IMO, 2004 & Anon., 2008) to cover the size range from 2 – 50 micron. This was in response to the tropical-oceanic character of the water, as derived from a detailed analysis. Our data and a literature research, made clear, that achieving the required intake numbers with organisms larger 10 micron only, would be virtually impossible considering the water quality in the region of intake (e.g. Gin et al., 2003). Further details can be found in the respective chapter 4.2.

Secondly, a cracked manifold was discovered in the main ballast line by the ship's crew. This would potentially contaminate every sample taken from the designated sampling points. Since repair was not possible before April 2015, a different sampling strategy was devised. Based on a recommendation in the U.S. ETV protocol (U.S. EPA, 2010; par. 5.3.2.5), a diaphragm pump was used for sampling on board the *Alfa Glory*.

It was found, that using a diaphragm pump for sampling was a practical solution, that guaranteed sample quality. Subsequently, all sampling was done with such a diaphragm pump.

3.2 Test run SII

Intake for this test run was on June 1st and it took about six hours to fill the ballast water tanks. At that time the ship was anchored in the harbour of Singapore, approximately two miles offshore (bunker anchorage). This is an open ocean type of water. Discharge was on June 11th. This resulted in a holding time of ten days. By then the ship had moved to the outer port limits and was anchored in international waters between Malaysia and Indonesia.

Figure 1: Intake location in the harbour of Singapore

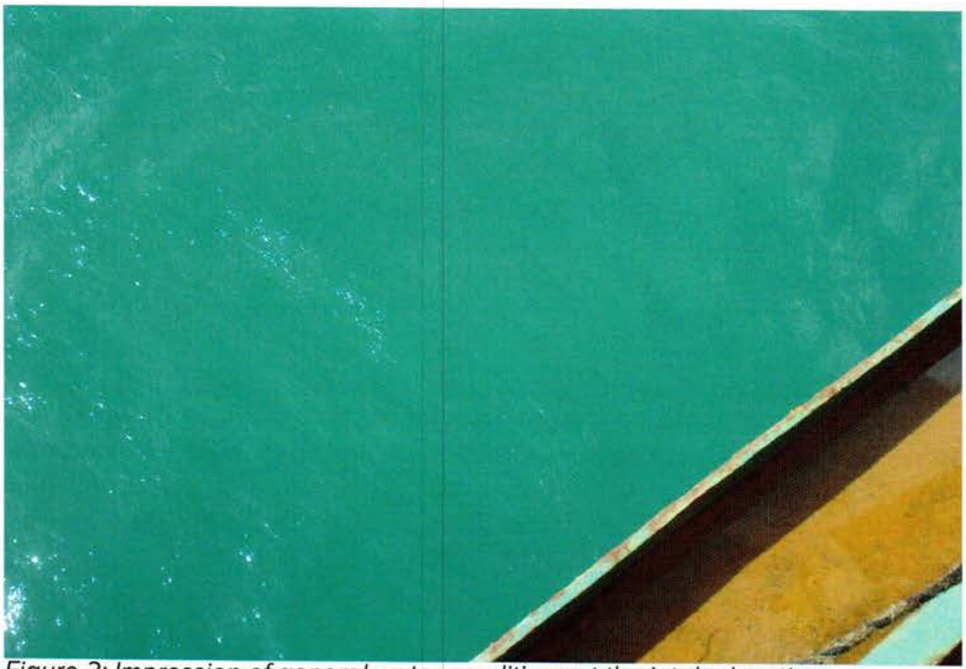

Figure 2: Impression of general water conditions at the intake location (tropical-oceanic)

Ballast tank 4 portside was used as treated tank. Ballast tank 2 starboard was used as control. Both tanks were filled simultaneously.

To improve the quality of the samples, and thus the counting, at the tropical oceanic conditions, sampling volume for control samples (organisms larger than 50 micron) was increased from 20 litres (Anon., 2008) to 40 litres (test protocol, MEA-nl 2014). Only the last sample was left at 20 litres, since tanks were full much quicker on intake than anticipated by the crew. After analysis, results showed, that there was no difference in numbers between the 20 and the 40 litre samples for this group of organisms.

3.3 Test run SIII

This test again consisted of an intake only. Intake took place on June 18th, in international waters between Malaysia and Indonesia. All samples for this intake were taken and analysed according to the specifications. Ballast tank 4 portside was designated as treated tank. Ballast tank 2 starboard was designated as the control tank. Both tanks were filled simultaneously.

A couple of days after intake, there was a change in ownership and management of the vessel In the transition period between the old and new owner, no testing was allowed. Therefore test run SIII could not be completed. Results from this intake were in-line with the data from test runs SI and SII.

3.4 Test run SIV

Intake for this test was on August 25th, southwest of Sri Lanka, en route to the Persian Gulf. Discharge was on September 3rd. This resulted in an effective holding time of 8.5 days. It was the second, completed (i.e. intake and discharge) test run performed. All samples for this test were taken and analysed according to the specifications. Ballast tank 4 portside was used as treated tank. Ballast tank 2 starboard was used as control. Both tanks were filled simultaneously.

Due to a technical failure of the ship's generators, treatment could not be performed according to specifications. This made the present test invalid. As a consequence, oxygen concentrations were never below 0.7 mg/l as shown by the in-tank data. Oxygen levels in the natural occurring oxygen minimum zone of the central Arabian Sea are < 0.1 mg/l (Böttger-Schnack, 1996). Similar values are reported for the eastern tropical Pacific OMZ (Saltzman and Wishner, 1997). This illustrates that no hypoxia and clearly no anoxia was achieved in the treated tank.

Even though both tanks were filled via the same ballast water pump, there was a remarkable difference in sediment load. Tank 4, the designated treated tank showed much higher TSS values at intake. The source/challenge water was oceanic and can be ruled out as source of this sediment load. It turned out that the sediment originated from within the ship's system, but why it affected tank 4 so much more than tank 2 was not fully understood.

Figure 3: Impression of general water conditions at the intake location (tropical-oceanic)


Figure 4: Turbid sample, taken from tank 4 on intake

3.5 Test run SV

Intake for this test run was on October 12th, in the early afternoon in the Gulf of Oman, in open water. Discharge was on October 18th. This resulted in an effective holding time of 5.5 days. Ballast tank 4 portside was used as treated tank. Ballast tank 2 starboard was used as control. Both tanks were filled simultaneously. All samples for this test run were taken and analysed according to the specifications.

3.6 Test run SVI

Intake for this test run was also on October 12th, but later in the evening, about 30 nautical miles away from the intake site for test run SV. Ballast tank 4 starboard was used as treated tank. Ballast tank 2 portside was used as control. Both tanks were filled simultaneously. This test run was entirely separated from the previous test run and should therefore considered as a full scale test run. This way of working ways discussed and accepted by the RO on forehand. Discharge was on October 18th, after the discharge of test run SV. This resulted in an effective holding time of 5 days. All samples for this test run were taken and analysed according to the specifications.

4. RESULTS

Results of the samples and analyses are given in this chapter. Normally, one would expect one value for intake for the control- and treated tank. However, during test run SI, it was agreed to make use of diaphragm pumps for each tank (see **chapter 3.1**). This resulted in two separate sets of data for intake from SII onwards.

4.1 Abiotic parameters

The abiotic parameters required by guideline G8 (Anon., 2008; Annex, Part 2, paragraph 2.2.2.9) to be reported (salinity, temperature, total suspended solids (TSS) and particulate organic matter (POC)) were measured as can be found in **table 3 until 6**:

Table 3: Salinity [PSU]

Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	41.4	na	42.3	na
SII	t10	35.3	34.6	35.6	34.9
SIII	na	37.2	na	38.8	na
SIV	t9	34.7	34.3	32.1	36.2
SV	t6	37.2	41.3	36.4	39.4
SVI	t6	41.7	41.1	40.7	40.2
min		35.3	34.6	35.6	34.9
max		41.7	41.3	40.7	40.2
Average values of Salinity in PSU. tX = day of discharge; na = not applicable					

Table 4: Water temperature [^{o}C]

Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	31.0	na	30.9	na
SII	t10	32.0	31.7	31.2	32.4
SIII	na	30.2	na	30.4	na
SIV	t9	29.4	30.0	29.4	29.9
SV	t6	30.7	31.2	31.2	31.5
SVI	t6	30.0	31.4	30.3	31.0
min		30.0	31.2	30.3	31.0
max		32.0	31.7	31.2	32.4
Average values of Water temperature in °C. tX = day of discharge; na = not applicable					

Table 5: Total Suspend Solids [TSS, mg/l]

Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	5.6	na	5.9	na
SII	t10	9.4	8.2	9.4	4.8
SIII	na	6.9	na	4.3	na
SIV	t9	12.8	27.7	151.1	17.3
SV	t6	3.6	4.4	5.1	5.9
SVI	t6	5.7	4.6	5.2	4.2
min		3.6	4.4	5.1	4.2
max		9.4	8.2	5.2	5.9
Average values of Total Suspend Solids (TSS) in mg/l. tX = day of discharge; na = not applicable					

Table 6: Particulate Organic Carbon [POC. mg/l]

Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	3.1	na	3.4	na
SII	t10	3.2	3.0	3.2	2.8
SIII	na	2.9	na	2.5	na
SIV	t9	3.7	5.7	18.5	4.1
SV	t6	2.1	2.2	2.4	2.6
SVI	t6	2.5	2.4	2.6	2.3
min		2.1	2.2	2.4	2.3
max		3.7	5.7	18.5	4.1
Average values of Particulate Organic Carbon (POC) in mg/l. tX = day of discharge; na = not applicable					

Analysis of all abiotic data confirmed that the challenge water for all test runs was typical tropical oceanic water (high water temperature, high salinity and low concentrations of TSS and POC). Intake values were as required by the Guideline G8 (Anon., 2008) and no differences were found between control and treated water at intake and discharge. This indicates that the BWT system does not affect abiotic parameters as was expected for TSS and POC, since no filtration step is used.

4.2 Biotic parameters

4.2.1 Organisms equal to or larger than 50 micron

Number of organisms per cubic metre can be found in **table 7**. All valid test runs were in compliance with regulation D-2 (IMO, 2004).

Table 7: Organisms ≥ 50 micron

Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	1,150.0	na	808.3	na
SII	t10	15,700.0	125.0	12,750.0	0.0
SIII	na	14,583.3	na	12,108.3	na
SIV	t9	1,058.3	100.0	2,091.7	19.0
SV	t6	8,275.0	316.7	8,325.0	6.7
SVI	t6	9,091.7	375.0	13,483.3	1.3
average		11,022.2	272.2	11,519.4	2.7
Min		8,275.0	125.0	8,325.0	0.0
Max		15,700.0	375.0	13,483.3	6.7
Average number of zooplankton-organisms per cubic-meter. tX = day of discharge; na = not applicable					

Intake values of organisms of 50 micron and larger in minimum dimensions, varied considerably but they met the required numbers at intake in all test runs (100 per m^3; Anon., 2008; Annex, par. 2.2.2.5). Copepods were usually the dominant organism-group, where the intake water of test run SV was dominated by Plathelminthes (flatworms). Control survival on discharge decreased significant, but met the requirements in all four test runs analysed. The incomplete treatment in test run SIV still induced 80 % extra mortality in this size class as compared to the control.

4.2.2 Organisms smaller than 50 but larger than 10 micron

Intake values for organisms greater than or equal to 10 micron, but less than 50 micron in minimum dimensions showed from first intake in Singapore, a typical size distribution for (sub-)tropical and oceanic waters. Smaller (i.e. less than 10 micron) sized organisms are dominant in numbers in these waters throughout the year rather than organisms in the larger than 10 micron size range. This is independent of seasonal influences, which usually affects overall numbers only moderately. The 2 to 10 micron size class of phytoplankton includes a large variety of potentially harmful algae causing frequently outbreaks of toxic algal bloom on a global scale.

From a biological point of view it is rather irrelevant whether a system treats an organism larger or smaller than 10 micron in cell size. Furthermore, for a statistically more reliable test run a sufficiently high cell number should be present. This could only be achieved by extending the range of cell size for intake numbers, but also for discharge. Treatment will not stop at a certain cell size but should affect the entire plankton community in the water to be treated.

Therefore, MEA-nl proposed to accept phytoplankton in a size range from 2 – 50 micron as challenge organisms for the purpose of these shipboard tests. That meant, that in practice, less than 10 viable organisms per ml in the size class from 2 – 50 micron, could be discharged water for a successful treatment.

The scientific instruments available at the laboratory of MEA-nl were capable of measuring numbers and viability of phytoplankton in the size range from 2 to 50 micron. This is an extension of the IMO relevant size class of 10 to 50 micron.

Lloyd's Register as RO accepted this proposal. Subsequently the extended size class was analysed accordingly, to assess the BWTS's performance.

4.2.2.1 Phytoplankton

Total biomass and viability of phytoplankton (including the size range from 2 to 50 micron), was analysed by measurement of the photosynthetic efficiency (PAM-fluorometry). Three samples were taken evenly distributed over the ballasting- and de-ballasting period. Analysis of the viability the phytoplankton community was conducted immediately on board of the vessel in replicate measurements.

Samples for phytoplankton enumeration were analysed using flow-cytometry and were stored in a refrigerator in the dark until further analysis. Flow cytometric analysis were done at the MEA-nl laboratory. Phytoplankton enumeration is analysed per ml. Results can be found in **table 8**.

Table 8: Total count of chlorophyl containing particles 2-50 micron number/ml

Testrun	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	206.8	na	171.3	na
SII	t10	1,673.8	96.9	1,581.3	97.7
SIII	na	2,102.4	na	1,746.0	na
SIV	t9	197.0	28.5	204.0	50.2
SV	t6	314.2	46.1	120.6	45.5
SVI	t6	143.8	33.0	163.0	39.0
Average		710.6	58.7	621.6	60.7
Min		143.8	33.0	120.6	39.0
Max		1,673.8	96.9	1,581.3	97.7
Average number of all chlorophyl containing particles per ml including dead ones. See text. tX = day of discharge; na = not applicable					

These numbers are total counts, i.e. living and dead cells. Results indicate that phytoplankton numbers were sufficiently high at intake and discharge. Viability information can be derived from the photosynthetic efficiency (Fv/Fm) measurements with the PAM (**table 9**). PAM measurements indicated that phytoplankton community at intake was in a photosynthetic active healthy status and therefore a viable condition. In all cases the index of the photosynthetic efficiency (Fv/Fm expressed as a ratio) exceeded the value of 0.3 which is considered healthy and viable.

A prolonged holding time in the dark up to 10 days negatively affected the viability of the phytoplankton community, resulting in a decline in the Fv/Fm. In general this decline was only moderate. The largest decline in viability was observed in the control tank of test run SII, with a holding time of 10 days.

A viability index (Fv/Fm) value below 0.1 is considered a critical cut-off point. Values below are considered to show a non-viable status of the phytoplankton cells. Values for the treated discharge water of test runs SV and SVI were still above 0.1. Additional incubation experiments were performed to examine the recovery in viability of representative water samples. Measurement indicated that there was no recovery of the phytoplankton community. Based on this supporting information it was concluded that all relevant test runs were in compliance with regulation D-2 (Anon., 2004) for the phytoplankton in the size range to 50 micron in minimum dimensions.

Table 9: Phytoplankton photosynthetic efficiency [Fv/Fm]

Testrun	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	0.39	na	0.50	na
SII	t10	0.48	0.15	0.47	0.05
SIII	na	0.40	na	0.34	na
SIV	t9	0.41	oos	0.40	0.07
SV	t6	0.34	0.40	0.33	0.13
SVI	t6	0.43	0.43	0.40	0.19
Average		0.42	0.33	0.40	0.12
Min		0.34	0.15	0.33	0.05
Max		0.48	0.43	0.47	0.19

Average Fv/Fm values as indication for viability. Values above 0.3 indicate a healthy population. Values between 0.3 and 0.1 indicate a stressed or dying population. Values below 0.1 indicate a dead population. tX = day of discharge; na = not applicable, oos = out of spec

4.2.2.2 Micro-zooplankton

Micro-zooplankton was analysed in the MEA-nl laboratory, using fixed samples. Analysis was done using inverted microscopy. Viability was based on intact cells.

Micro-zooplankton was not abundant at intake and discharge (**table 10**). With the exception of the control of test run SV, micro-zooplankton decreased both in the control and treated samples during holding time.

Although samples after treatment indicate that the regulation D-2 was met, there is no clear distinction between control and treated at discharge for most test runs.

Table 10: Micro-zooplankton

Test run	tX	Control t0	Control tX	Treated t0	Treated tX
SI	na	0.05	na	0.06	na
SII	t10	2.19	0.04	nd	1.03
SIII	na	2.43	na	1.87	na
SIV	t9	2.12	0.57	0.68	0.69
SV	t6	1.86	2.72	0.87	0.43
SVI	t6	0.99	0.11	0.60	0.34
average		1.68	0.96	0.74	0.60
Min		0.99	0.04	0.60	0.34
Max		2.19	2.72	0.87	1.03
Average number of micro-zooplankton organisms per ml. tX = day of discharge; na = not applicable, nd = not determined					

4.2.3 Indicator microbes

Two different sets of bacteria samples were taken and analysed for human pathogens and for heterotrophic bacteria.

4.2.3.1 Human pathogens

Samples for human pathogens, i.e. the indicator microbes *Escherichia coli, Vibrio cholera* (O1 and O139) and Enterococci as defined in Regulation D-2 (IMO, 2004) were analysed by certified laboratories from test runs SII, SIII, SV and SVI. All samples, i.e. intake and discharge water of both control and treated, contained no detectable human pathogens (**table 11**).

Table 11: Indicator microbes

E. coli					
Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	nd	na	nd	na
SII	t10	nd	< 1	nd	<1
SIII	na	< 1	na	< 1	na
SIV	t9	nd	nd	nd	nd
SV	t6	< 1	< 1	< 1	< 1
SVI	t6	< 1	< 1	< 1	< 1

Enterococci					
Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	nd	na	nd	na
SII	t10	nd	< 1	nd	< 1
SIII	na	< 1	na	< 1	na
SIV	t9	nd	nd	nd	nd
SV	t6	< 1	< 1	< 1	< 1
SVI	t6	< 1	< 1	< 1	< 1

Vibrio Cholerae					
Test run	tX	Control-t0	Control-tX	Treated-t0	Treated-tX
SI	na	nd	na	nd	na
SII	t10	nd	< 1	nd	< 1
SIII	na	< 1	na	< 1	na
SIV	t9	nd	nd	nd	nd
SV	t6	< 1	< 1	< 1	< 1
SVI	t6	< 1	< 1	< 1	< 1

Analysis results from the external laboratories for the indicator microbes *Escherichia coli, Vibrio cholera* (O1 and O139) and Enterococci. tX = day of discharge; nd = not determined, na = not applicable

Samples of test run SIV were analysed for reference only with an experimental device, the SpeedyBreedy™ from Bactest. With that the presence of vibrios (in this case referring to the generic group and not the indicator microbes O1 and O139 only) was measured on intake for both control and treated tanks. The discharge samples of this series showed the presence of vibrios in the control discharge water but none in the treated discharge water. The SpeedyBreedy™ was also taken on board for test runs SV and SVI. In line with the results from the certified laboratory, no indicator microbes were detected at intake.

4.2.3.2 Heterotrophic bacteria

The second set of samples was taken for heterotrophic bacteria. This term does not refer to a specific type of bacteria, but encompasses all bacteria that are dependent on the intake of organic material as a source for growth.

A ship and a ballast tank are not a sterile environment, so bacteria will always be present in high numbers. That is also true for a ballast tank that is treated successfully. Therefore IMO did not define a discharge standard for heterotrophic bacteria, but rather for the indicator microbes of concern to human health discussed above.

However, bacteria numbers offer a wealth of information on the biological state of the water. Therefore these samples were taken and analysed in addition to the indicator microbes.

The samples for heterotrophic bacteria numbers taken by MEA-nl were preserved with a fixative and therefore represent only total rather than a differentiation between viable and non-viable bacteria.

Table 12: Heterotrophic bacteria number/ml

test run	tX	Intake-t0	Control-tX	Treated-tX
SI	na	nd	na	na
SII	t10	7,2E+05	3,0E+05	7,0E+05
SIII	na	nd	na	na
SIV	t9	nd	nd	nd
SV	t6	1,6E+06	8,9E+05	4,6E+05
SVI	t6	1,4E+06	8,8E+05	6,4E+05
average		1,3E+06	6,9E+05	6,0E+05
min		7,2E+05	3,0E+05	4,6E+05
max		1,6E+06	8,9E+05	7,0E+05

Average number of heterotrophic bacteria per ml. tX = day of discharge; nd = not determined, na = not applicable

Table 12 shows total numbers, i.e. living and dead bacteria combined, and gives no information on viability of the bacteria. As can be seen, numbers decreased during holding time, both in the control and treated tanks. From this data, it may be derived that the BWT system does not affect total numbers of bacteria significantly.

4.2.4 Plankton diversity challenge water

Although no detailed data are included in the report the biodiversity of the plankton in the organisms larger than 50 micron as well as in the 10 to 50 micron size range, was in accordance with the Guideline G8 (Anon., 2008) (Five different species of at least of three different phyla).

5. SUMMARY AND & CONCLUSIONS

The test runs SII, SV and SVI were valid and showed proper results.
Based on the technical- and operational issues on board the vessel, test run SI, SIII and SIV were invalid.

At three moments, there were deviations from the original test protocol. Those deviations were discussed with Coldharbour and Lloyd's Register and accepted as described in this report. They included:

1. Extension of the size class for viable organisms from 10 – 50 to 2 – 50 micron in minimum dimensions (see par. 3.10 and 4.2.2);
2. The use of diaphragm pumps for sampling at intake and at discharge for each individual tank (see par. 3.1); and
3. An increased sample volume for zooplankton (see par. 3.2)

Abiotic data confirmed that the challenge water for all test runs was typical tropical oceanic water. No differences were found between control and treated water at intake and discharge. This indicates that the BWT system does not affect abiotic parameters as was expected for TSS and POC, since no filtration step is used.

Concerning the biotic data, The system proved operational under tropical conditions and successfully treated 27,000 cubic metres of water during the three valid test runs. As can be seen in sub-chapter 4.2, the BWT system did comply with Regulation D-2 (Anon., 2004), if operated according to its specifications. No hard conclusion can be drawn to the performance of the BWTS regarding the treatment of human pathogens. Both, intake- and discharge numbers were too low.

The system proved to be effective in preventing potential invasive species in ballast water and sediment. All the data collected are robust and cover all required parameters. Sampling and analysis could be performed up to standard at all times.

In conclusion these results show that the Coldharbour Marine BWTS is capable of meeting the discharge criteria in accordance with regulation D-2 (IMO, 2004) in accordance with the requirements of the Guideline G8 (Anon., 2008) for shipboard tests under operational conditions on board of a VLCC.

6. REFERENCES

6.1 List of references

Anonymous (2004) IMO International convention for the control and management of ship's ballast water and sediments. BWM/CONF/36, 16 February 2004

Anonymous (2008) Guidelines for approval of ballast water management systems (G8). Annex 2 Resolution MEPC.174.58)

Böttger-Schnack, R. (1996) Vertical structure of small metazoan plankton, especially non-calanoid copepods. I. Deep Arabian Sea. Journal of Plankton Research 18: 1073-1101.

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MEA-nl (2014) Testprotocol for the shipboard verification of the ballast water treatment technology Coldharbour Marine Ltd.

Saltzman, J., K. F. Wishner (1997) Zooplankton ecology in the eastern tropical Pacific oxygen minimum zone above a seamount: 1. General trends. Deep-Sea Research 44(6): 907-930.

U.S. Environmental Protection Agency (2010) Generic protocol for the verification of ballast water treatment technology. EPA/600/R-10/146, September 2010

6.2 List of standard operation procedures (SOP's) of MEA-nl

SOP-306	salinity, temperature, pH,dissolved oxygen and turbidity; 30.09.2013; version-number 1.1
SOP-308	Dissolved organic carbon and inorganic nutrients; 24.01.2014; version-number 1.1
SOP-309	TSS, POC, MM and dissolved nutrients; version-number 1.1 as of 19.05.2014
SOP-311	Human Pathogens; 24.01.2014; version-number 1.1
SOP-316	Organisms < 10 µm bacteria FCM; 26.09.2012; version-number 1.0
SOP-317	Organisms 10-50 µm FCM; 26.09.2012; version-number 1.0
SOP-318	Organisms 10-50 µm PAM; 26.09.2012; version-number 1.0
SOP-319	Organisms 10-50 µm Microscopy; 26.09.2012; version-number 1.0
SOP-320	Organisms > 50 µm; 26.09.2012; version-number 1.0
SOP-322	Organisms < 10 um phytoplankton FCM; 26.09.2012; version-number 1.0
SOP-326	Incubation experiments; 26.09.2012; version-number 1.0

6.3 List of figures

6.4 List of tables

6.5 Glossary of terms

Accreditation	The meaning assigned to it by Regulation (EC) No. 765/2008.
Active Substance	A substance or organism, including a virus or a fungus that has a general or specific action on or against Harmful Aquatic Organisms and Pathogens.
Amendment	A change to a specific verification protocol or a test plan prior to performing the verification or test step.
Ambient Populations	The biological organisms, including bacteria, protists, and zooplankton that are naturally occurring in the water at the test facility location.
Ballast Water Management Plan	The document referred to in Regulation B-1 of the Ballast Water Management Convention (BWMC) describing the ballast water management process and procedures implemented on board individual ships.
Ballast Water Treatment Equipment	Equipment which mechanically, physically, chemically, or biologically processes, either singularly or in combination, to remove, render harmless, or prevent the uptake or discharge of Harmful Aquatic Organisms and Pathogens within Ballast Water and Sediments. Ballast Water Treatment Equipment may operate at the uptake or discharge of ballast water, during the voyage, or at a combination of such events.
Challenge Water	Water supplied to a treatment system under testing. Challenge water must meet specified ranges for densities of living organism and water-quality parameters and is used to assess the efficacy of the treatment equipment under full-scale operational conditions.
Cyst	The dormant cell or resting stage of microalgae, heterotrophic protists, and metazoans, including, but not limited to, cysts of dinoflagellates, spores of diatoms, cysts of heterotrophic protists, and cysts of rotifers.
Deviation	A change to a specified verification protocol or a test plan prior or during the verification or performance of a test step.
Effluent	The discharge of treated water produced by a ballast water treatment technology or system.

Equipment	The ballast water treatment system, defined as either a package or a modular system, which is to be tested for Type Approval.
General verification protocol (GVP)	The description of the principles and the general procedure to be followed by the ETV pilot programme when verifying an individual environmental technology.
In-Line Treatment	A treatment system or technology used to treat ballast water during a normal flow of ballast during intake or discharge.
Land-based Testing	A test of the ballast water management system (BWMS) carried out in a laboratory, equipment factory or pilot plant, either on land or on a test barge or test ship.
Manufacturer	A company or individual that manufactures, assembles, or sells ballast water management systems.
Monitoring Equipment	The equipment installed for the assessment of an effective operation of the Ballast Water Treatment Equipment.
Performance Data	Efficacy of removal and data on effluent concentration for core and supplemental parameters for a given set of challenge conditions.
Performance claim	A set of quantified technical specifications representative of the technical performance and the potential environmental impacts of a technology in a specified application and under specified conditions of testing or use (operational parameters).
Precision	The degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. Precision is usually expressed as standard deviation, variance, or range, in either absolute or relative terms (NELAC, 1998).
Protocol	A written document that clearly states the objectives, goals, scope, and procedures and logistics for the study of a particular group of similar technologies. A protocol shall be used for reference during manufacturer participation in the verification testing program.
Quality Assurance Project Plan (QAPP)	A written document that describes the implementation of quality assurance and quality control activities during the life cycle of the verification process (also see Test/quality assurance plan).

Representativeness	The degree to which data accurately and precisely represent a characteristic of a population.
Sensitivity	The capability of a test method or instrument to distinguish between different levels (e.g., concentrations) of a variable of interest.
Standard Operating Procedure (SOP)	A written document containing specific instructions and protocols to ensure that the requirements for quality assurance are maintained.
Test Cycle	One intake/discharge cycle (including appropriate holding periods) designed to gather data on treatment efficiency.
Test Facility	A site that provides the necessary infrastructure, equipment and (scientific) personnel to complete the land-based testing for Type Approval. The facility may be part of the Testing Organisation or may be independent from the Testing Organisation, but in any case shall be totally independent from manufacturers of technologies testing at their site.
Test/Quality Assurance Plan (TQAP)	Also called a Quality Assurance Project Plan (QAPP), is a written document that describes the procedures for conducting a test or study according to the requirements of the verification protocol for the application of a particular ballast water treatment system or technology. At a minimum, the TQAP shall include detailed instructions for sample and data collection, sample handling and preservation, precision, accuracy, goals, and requirements for quality assurance and quality control relevant to the particular site.
Testing Organisation (TO)	An organisation qualified to conduct studies and testing of ballast water treatment technologies in accordance with the appropriate protocols and TQAPs.
The Convention	The International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO).
Treatment Rated Capacity (TRC)	The maximum continuous capacity expressed in cubic metres per hour for which the BWMS is type approved. It states the amount of ballast water that can be treated per unit time by the BWMS to meet the standard in Regulation D-2 of the Convention.

Verification	Means the provision of objective evidence that the technical design of a given environmental technology ensures the fulfilment of a given performance claim in a specified application, taking any measurement, degree of uncertainty and relevant assumptions into consideration.
Verification Organisation (VO)	The party responsible for overseeing development of the TQAP, overseeing the testing activities in conjunction with the Testing Organisation, and overseeing the development and approval of the Verification Protocol, the Report and the Verification Statement for the ballast water treatment system. As certification is a task of a national administration, this is either a National Administration or a Classification Society authorised by the NA.
Verification Report	A detailed report on the testing results of a particular technology according to an approved Test /Quality Assurance Plan and conducted under the ETV/GTV protocol. The report is typically prepared by the Testing Organisation and contains a description of the test facility, photographs of the technology being tested, applied methods and procedures and a presentation of analysed data including all QA/QC data obtained during the test. Appendices include raw data sets and lab audit information, TQAP, O&M Manual and other relevant information.
Verification Statement	An executive summary of the verification report. A summary of the data will be part of Type Approval Certificate.
Verification Test	A complete test of a treatment system, following a well-defined TQAP which includes enumeration of ambient and test populations in the challenge water to determine the efficacy of the technology.
Viable	According to the IMO G8 Guideline, "organisms and any life stages thereof that are living". This differs to the scientific definition, "organisms which are capable of reproducing".
Vital	Essential to the continuation of life.

6.6 Abbreviations and acronyms

AC	Acetic acid
BE	Biological efficacy
DBP	Disinfection By-Products
BWM	Ballast Water Management
BWMS	Ballast water management system(s)
m^3	cubic meter, equivalent to 1000 litres
DOC	Dissolved organic carbon
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environment Protection (http://www.gesamp.org/)
GESAMP-BWWG	GESAMP-Ballast Water Working Group
GVP	General Verification Protocol (EU Environmental Technology Verification pilot programme)
IMO	International Maritime Organisation (http://www.imo.org)
µg/l	Micrograms per litre
mg/l	Milligrams per litre
MM	Mineral Matter
nd	not determined
NEN-EN-ISO 9001	Quality Management Systems – Requirements (ISO 9001: 2008)
NTU	Nephelometric turbidity unit
PAA	Per Acetic Acid
PSU	Practical salinity units
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
QMP	Quality Management Plan
SOP	Standard Operating Procedure
TA	Type Approval
TO	Testing Organisation
TQAP	Test/Quality Assurance Plan
TSS	Total Suspended Solids
VO	Verification Organisation

ANNEX 1 – QUALITY ASSURANCE

MEA-nl is a test organisation with clear policies and procedures described in the Quality Management System based on NEN-EN-ISO 9001:2008 (further referred as ISO 9001). Scope of the certification is:

"To perform tests for certification of ballast water management systems"

June 6, 2013, the certificate is issued on behalf of the United Kingdom Accreditation Service (UKAS) by Lloyd's Register Quality Assurance.

Quality is crucial for MEA-nl at all operational levels. All products and services are based on the same quality according to ISO 9001. MEA-nl assures that all analytical methods done at external labs are done conform at least the same quality requirements as at MEA-nl.
The present Quality Management System of MEA-nl will be changed in accordance with the requirements of ISO-17025.

Quality of measurements and data acquisition is guaranteed by having a standard operating procedure (SOP) for every method and measurement needed to verify the performance of a BWM system. SOPs are part of our Quality Management System, assuring that measurements are transparent and easily reproducible, irrespective of which trained personnel is performing the measurement. Methods are all based on well-established scientific procedures. All tests and measurements needed to verify a BWM System are done by experienced personnel. Test results will be recorded at forms or notebooks as specified in SOPs.

Besides data in general, non-conformities such as deviations and out of specs are crucial to assure data reliability. Based on MEA-nl related activities, different non-conformities occurred during the verification tests of the BWM System of Coldharbour . All these non-conformities are solved following our Quality Management System and did not affect the results of the verification tests and results of the BWM System of Coldharbour.

MEA-nl also aims to enhance customer satisfaction through an effective Quality Management System, including processes for continual improvement of the system and the assurance of conformity to customer requirements. Customer satisfaction is monitored by the contact person of every customer of MEA-nl. During the whole process of shipboard testing of the Coldharbour BWM system there was continuously contact between Frank Fuhr and the representative(s) of Coldharbour to meet the customer's requirements. No complaints were received during the whole process until this moment. Based on this report a last customer satisfaction verification will be done to finalize this process and to assure that all procedures and processes were done conform the customer requirements. With this information MEA-nl tries to reach information for future improvement and development.

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